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MORPHOLOGY AND PARASITISM OF *ACROTHECIUM PENNISETI* N. SP.

(A NEW DISEASE OF *PENNISETUM TYPHOIDEUM*)

BY

MANORANJAN MITRA, M.Sc

First Assistant to the Imperial Mycologist

IMPERIAL AGRICULTURAL RESEARCH INSTITUTE
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AGRICULTURAL RESEARCH INSTITUTE, PUSA

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MORPHOLOGY AND PARASITISM OF *ACROTHECIUM PENNISETI* N. SP.

(A NEW DISEASE OF *PENNISETUM TYPHOIDEUM*.)

BY

MANORANJAN MITRA, M.Sc.,

First Assistant to the Imperial Mycologist.

[Received for publication on 2nd June, 1920.]

1. Introduction.

Pennisetum typhoideum Rich. (vern. Bajra) is a tall, erect grass of African origin. The total area under it in India is about 13,320,250 acres, yielding 2,197,425 tons of grain. In the order of importance it takes the fourth position among the cereals, following rice, wheat and *Sorghum*.[1] It is very commonly cultivated in North-West, Central and Southern India, as a cereal food and also for fodder. In the Bombay Presidency the area occupied by it is considerably over 4 million acres, and in many parts it forms the staple food of the people. It is generally sown in June, and the crop ripens in September. In Sind and Rajputana, it is the most important *kharif* crop. In Madras and the Punjab, it is grown to a very great extent and is used both as a staple food and fodder.

The number of fungus diseases hitherto reported as attacking this crop in India is not large : *Sclerospora graminicola* (Sacc.) Schroet., *Puccinia Penniseti* Zimm., and *Tolyposporium Penicillariæ* Bref. being the chief.[2]

A parasitic species of *Acrothecium* is very common on Bajra at Pusa, which occurs every year and seems to cause considerable damage. It has never been recorded before, though its presence is noticed on diseased ears occasionally sent to Pusa from outside. Thus the fungus was found on ears sent to the Imperial Mycologist by the Superintendent, Government Farm, Alibag (Bombay), in 1919.*

The genus *Acrothecium* is a Deuteromycete belonging to the group Dematiaceæ, Phragmosporæ. The chief characteristic of this genus is that the

[1] *Agri. Statistics of India*, 1918–19, Vol. I.

[2] Butler, E. J. "Fungi and Disease in Plants," 1918, pp. 218—226.

* Since this paper was submitted for publication, the writer has found it to be very common at Cawnpore, Hansi, Amritsar, Gurdaspur, Lahore and Lyallpur.

conidiophore is simple, erect, and of dark colour, and bears a group of two to many-celled, dark-coloured conidia at its tip.

There are several species of *Acrothecium* recently found at Pusa on various hosts, *viz.*, Jowar (*Sorghum vulgare*), maize (*Zea Mays*), rice (*Oryza sativa*), and wild grasses such as *Andropogon* and *Panicum*, and all these have been brought into culture and are under investigation.

2. The disease: Its description.

At Pusa, the organism appears on leaves, leaf-sheaths, and on ears. Since it is most common on the leaves, the disease may be called "leaf spot or leaf blight of *bajra*."

(*a*) *Appearance on the leaves.* In the beginning the infected leaves show small yellowish-brown spots which gradually spread more in the longitudinal direction and become oval or oblong. The centre of the spot soon changes to a dirty brown colour, around which the margin remains yellow. Sometimes the spots coalesce and form irregular patches. They are most frequent towards the edge of the leaf, and in cases where they are situated near each other they soon run together, unite and form a big spot, killing the tissue along the margin of the leaf and gradually extending towards the midrib. Frequently both margins are infected and the whole leaf is gradually killed. The spots occur on the midrib also, especially on those of young leaves and, in severe cases, even on the leaf-sheaths. Very often the infection begins from the tip of drooping leaves and goes on increasing and extending towards the base, either along the margin or along the midrib or both together. The infected portion of the leaf becomes brittle with age. The disease begins from the lower leaves, then gradually spreads and attacks the upper leaves. In the lower leaves the infection begins from the tip of the drooping leaves which either touch the soil or are very near to it.

From the observation made at Pusa in 1919, it has been found that the disease makes its appearance in June on the lower half-dead leaves of young plants, but is associated with many other saprophytic organisms. With the increase of rainfall in July, it becomes more and more prominent and is found on the lower leaves of a large number of plants. In August and September it is also found on the upper leaves. Towards the middle of September (in 1919), almost all *bajra* plants were found to be more or less affected. The spread of the disease is facilitated by the moisture which the tip and margin of the leaves retain during the monsoon on account of their being a little up-turned (Plate I, figs. 1 and 2).

(*b*) *Appearance on the ears.* Not only leaves and leaf-sheaths but the ears also are attacked by this fungus (Plate I, fig. 3). The glumes and palæ of the

flowers show the presence of this fungus. The infection begins from the tip of the floret and gradually the whole spikelet gets infected. From the first scattered infected spikelets the attack spreads to those in the neighbourhood, and in a badly infected ear very few normal grains are formed. When once the ear is weakened by the disease, other fungi also make their appearance. An infected flower shows a tuft of black mould at its tip, and inside mycelium is found in the ovary and anthers. Plate I, fig. 3, shows an ear which was inoculated in a few places.

3. The economic importance.

The organism appears to cause considerable damage to the leaves and ears of *bajra* and affects the growth and production of sound and healthy grains. It hinders the assimilation of carbon dioxide by the leaves and thus the plant is starved to some extent. Further information as to the amount of damage it does to the crop in years when the attack is severe is required.

4. The morphology of the fungus on the host.

Mycelium. The mycelium consists of septate hyaline hyphæ which ramify in the tissues of the host. It is olive brown in colour where conidiophores are given off. It is both intra and inter-cellular, and is found in all parts of the diseased tissues, even in the cells of the endodermis, sclerenchyma and vessels. Haustoria are not present.

Conidiophores (Plate II, figs. 1 and 2) are present in greater number in the central dead portion of the spot and they gradually get lesser and lesser towards the margin. They come out from the stomata of the leaf blade and midrib. They are amphigenous, solitary, fasciculate, rigid, erect, straight or slightly nodulose or bent ; simple and 3—5 septate. Rarely they are forked near the tip (Plate II, fig. 2*a*). In colour they are olive brown to dirty brown with paler tips which are either swollen or flexuous. The base sometimes is also swollen. They measure 68·4—154 μ long and 5·8—8·7 μ broad.

Conidia (Plate II, fig. 3) are clavate, pear-shaped, or elongated, straight or slightly bent, thick-walled, 2-3 septate, light olive-brown to dirty brown in colour, end cells lightly coloured and middle one broader and darker. They are constricted at the septa and are borne at the tip of the conidiophore in groups of 2—5. Very rarely conidia on the sides of the conidiophore below the tip are noticed. They measure 25—41·8μ × 12·5—20μ.

5. Cultures.

Pure cultures were obtained several times from typical leaf spots by single spore isolations from poured plates. The spores germinate freely

in glucose agar and can be picked up and transferred to culture tubes.

(*a*) *Germination of conidia and growth of fungus in distilled water.* The spores readily germinate in distilled water. Fresh spores begin to give out germ tubes after one or two hours from placing in water (Plate II, fig. 3*a*; and Plate III, figs. 6—10). Germ tubes protrude either from one end or from both. The tube is swollen at the base, hyaline and sparingly septate in the beginning. Sometimes two germ tubes come out from two points in one extremity (Plate II, fig. 6) or a single germ tube which at once bifurcates. The hyphæ elongate, branch and anastomose, and form a net-work of mycelium which only sometimes produces spores in water. These spores are smaller in size than those produced in culture tubes. In hanging drop cultures either a very few or none at all germinate. Probably this has something to do with the supply of oxygen. Frequently no spore formation takes place in closed culture tubes, but when a little of the culture is placed on a slide with a drop of distilled water and is incubated, a free production of conidiophores and conidia results. This is probably due to a combination of free supply of oxygen and moisture together with a lesser amount of food. Sometimes, instead of the formation of conidia the tip of the conidiophore grows out into a hypha (Plate II, fig. 7).

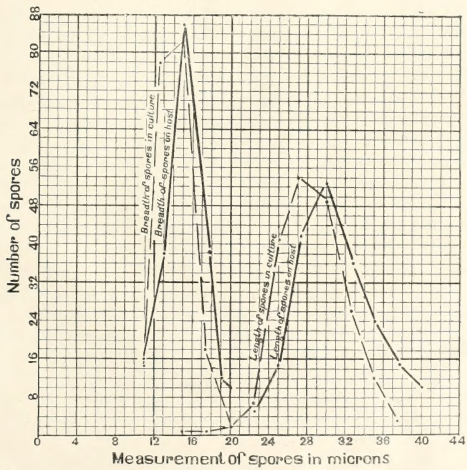
(*b*) *Morphology of the fungus in culture.* In ordinary nutrient glucose agar the fungus grows profusely. In the beginning the aerial mycelium is pale but it gradually changes to light pink and then to greyish brown. With the increased growth of the fungus the medium turns dark brown. The submerged and creeping hyphæ are hyaline to light greyish brown in colour. The mycelium is septate with cells up to 7·5 μ in diameter, and goes on branching and anastomosing and producing a large number of conidiophores at right angles to the main hyphæ or at their tips (Plate II, figs. 4—6).

The conidiophores are deeper in colour but in diameter generally they are of the same size as the sterile hyphæ. There is not much difference in the characters of the conidiophore as formed in culture from those already described on the host. They generally bear 2—5 conidia at their tips (Plate II, figs. 4—6) but sometimes only one and occasionally more than 5 are seen. In the beginning a small bud-like projection appears, this soon increases in size; septa develop and thus a conidium is formed. At first one appears, then another, and so on, till a group of 2—5 is formed.

Frequently, on some media, a second set of conidia is formed below the tip, on the sides of the conidiophore, and often the conidiophore, after producing a group of conidia at the tip, resumes its growth, and produces another group, while the first group either remains adherent or gradually gets detached. In

the latter case the conidiophores are quite long, and are angularly bent and nodulose at the point where there has been a formation of conidia. This formation of secondary sets of conidia takes place in old cultures and, to a somewhat lesser extent, in cultures that are taken out of the tube and incubated for some days on slides. When the conidiophore is formed at the end of a hypha (Plate II, figs. 4, 6), it is distinguished from other hyphæ by its colour which turns distinctly deeper from a particular septum.

The characteristics of conidia have already been described, but those produced in culture are slightly smaller in size than those found in the field. The accompanying curve (Text-figure) is drawn to compare the length and breadth of conidia in nature and in artificial culture. The conidia generally are 2–3 septate, but in culture sometimes uniseptate ones are formed (Plate III, fig. 11)



The formation of chlamydospores takes place in most of the cultures. The cells of a hypha swell up singly or sometimes in a long line, become spherical or oval in shape, later on become thick-walled and, as the culture gets old,

turn brown or greyish brown in colour. These chlamydospores either remain united in a chain or separate, and often germinate to give rise to new hyphæ (Plate III, figs. 1—3).

(c) *Growth of fungus on different culture media.* A comparative study of the growth of the fungus has been made on 20 different culture media. Two tubes of each medium were inoculated on the same day and all these tubes were kept at the room temperature which was between 20 and 23°C. Media of known reaction were employed and the reaction of each is indicated with the cultural description. All the media used were acid in reaction, ranging from + 2 to + 9 Fuller's scale. The composition of the media is given at the end in the Appendix (p. 72).

The fungus can grow well on most media tried, but does best on potato juice agar, wheat broth agar, nutrient glucose agar and French-bean agar. In all of these growth was profuse and the mycelium spread even on to the walls of the tubes. Conidia were formed in large number except on French-bean agar, on which only a few spores were observed. Of the above four media, potato juice agar and wheat broth agar were the best, both as regards the growth and production of spores. Besides these a fair growth was also found on the other meal agars described below. The growth was poor on glycerine agar, cellulose agar and on corn leaf juice agar. Details of the growth on all the media are given below.

1. *Potato juice agar* (+6 Fuller's scale). The growth is best on this medium. Woolly aerial mycelium fills three-quarters of the tube in a few days and spreads on the walls. The colour in the beginning is light grey, but later on it changes to dark grey. There is a slight tinge of pink present on the sides where the medium and the tube meet. In six days the growth is very profuse and the tube is quite full ; the mycelium becomes darker and the medium gradually turns yellowish in colour. The creeping hyphæ are subhyaline and in older cultures they become olive brown. They are up to 7·5μ in diameter. Thick-walled dark brown chlamydospores are also formed. Spore formation is very free and sometimes, in the older cultures, the conidia develop below the tip of the conidiophores. They measure 15—20μ × 10—12·5μ.
2. *Thaxter's hard potato agar* (+6 Fuller's scale). Growth poor at first but later on fairly good. The medium becomes slightly yellowish near the margin in the beginning and then turns darker gradually. Aerial growth is moderate, dark olive grey in colour. The appearance of the growth is somewhat like that on nutrient

glucose agar, but less luxuriant. Conidia numerous, sometimes on the sides of the conidiophores, 22·5—30μ × 12·5—16·2μ in diameter. Chlamydospores are also formed.

3. *Wheat broth agar*. Growth profuse, nearly as good as No. 1. The medium in the beginning is slightly pink in colour along the margin, but the main portion is grey. After a few days, the marginal pinkish colour turns slightly yellow and the main portion darkens. Aerial mycelium is produced in a sufficiently large amount and almost fills the tube. Hyphæ subhyaline to greyish brown and up to 7μ in diameter. Spore formation copious and begins when the culture is only two days old. Conidia 2–5 at the tip of conidiophore, but often only one and occasionally up to 8 are noticed. Secondary sets of conidia are also frequently formed. They measure 25—35μ × 12·5—15μ.

4. *Wheat meal agar* (+2 Fuller's scale). The growth of the fungus is crowded or compact. First the medium gets pink towards the margin, then gradually changes into light greenish grey. This colour predominates for some time in the whole medium and then, as the culture ages, it changes into dark yellow. Hyphæ subhyaline to dark grey and up to 7·5μ in diameter. Spore formation fair and conidia 25–32·5μ × 12·5–15μ in diameter.

5. *French-bean agar* (+8 Fuller's scale). A good growth with abundant aerial mycelium almost filling the tube within 7 or 8 days. The medium is first grey, then dark in colour. Hyphæ remain either sterile or very few spores are formed, but there is a great development of chlamydospores. The conidia are 27·5μ long and 15μ broad.

6. *Nutrient glucose agar* (+5 Fuller's scale). Copious woolly aerial growth. Aerial hyphæ greyish brown in colour. The medium in the beginning becomes light pink and this later on turns into dark brown. Aerial hyphæ after some days change into olive brown. Spore formation begins when the culture is 3 or 4 days old and conidia are formed in large numbers. They measure 25—37·5μ × 12·5—20μ. Besides this formation of spores, chlamydospores of a dirty brown colour are produced in fairly large numbers.

7. *Rice meal agar* (+4 Fuller's scale). The growth is fair and slightly pink in colour as in the last medium. With age, the hyphæ become darker in colour. The margin of the medium changes to pinkish yellow. Spore formation poor. The conidia measure 22·5—37·5μ × 10—16·2μ.

8. *Jowar meal agar* (+2 Fuller's scale). Growth as in the last. After a few days the medium becomes light yellow in colour. Few chlamydospores occur. Conidia are formed in fairly large numbers. They measure 22–37·5μ × 12·5—17·5μ.

9. *Jowar leaf juice agar*. The growth is almost submerged, but there is abundant spore formation. Conidiophores are often angularly bent and nodulose. The conidia measure 17·5–30μ × 12·5—15μ.

10. *Barley meal agar* (+3 Fuller's scale). The growth is poor. The hyphæ are dark grey. The medium is dark yellow near the margin. Abundant spore formation takes place and the conidia measure 25–32·5μ × 12·6–16·2μ.

11. *Litmus lactose agar* (+6 Fuller's scale). The growth is poor. With age, the red colour of the litmus changes into blue and then darkens, showing that the medium is becoming alkaline in reaction. Few chlamydospores are formed. Spore formation is poor and the conidia measure 22·5–32·5μ × 10–16·2μ.

12. *Dextrose agar* (+2 Fuller's scale). Growth is poor. Aerial mycelium is little and of grey colour. Old cultures become slightly pink. No chlamydospore formation. Conidium formation poor. Conidia measure 25—30μ × 12·5—15μ.

13. *Oat meal agar* (+2 Fuller's scale). Growth fair and crowded. No chlamydospore formation. Spores form in large numbers and measure 22·5–32·5 × 10–16·2μ.

14. *Glycerine agar* (+6 Fuller's scale). Very poor growth. The hyphæ are almost submerged and the medium turns pink in colour; later on the hyphæ become grey. A good many chlamydospores are formed. Conidium formation fair and conidia measure 22·5–32·5μ × 10–15μ.

15. *Cellulose agar* (+9 Fuller's scale). Very poor and submerged growth in this medium. For the first few days there was little growth and a few chlamydospores were produced. Spore formation is fair ultimately and in measurement the conidia are 25–37·5μ × 12·5–17·5μ.

16. *Starch agar* (+4 Fuller's scale). The growth in the beginning is poor, but after a few days it improves. The growth is sub-aerial with some submerged. The medium becomes dark grey. No chlamydospore formation takes place. Conidia in fairly large numbers and measure 20–30μ × 10–16·2μ.

17. *Cocoa meal agar* (+2 Fuller's scale). The growth is quite good and is pink in the beginning, but later on it changes into dark grey.

The medium gradually turns yellow in colour. Spore formation copious as in wheat broth agar. In measurement the conidia are 22·5—32·5 $\mu \times 10$—15μ.

18. *Corn leaf juice agar* (+3 Fuller's scale). The growth is bad and almost entirely submerged. Very few spores are formed.
19. *Naegelis nutrient solution.* Growth very poor. Very little aerial mycelium.
20. *Cohn's nutrient solution.* As in the above, here also growth is poor with very little aerial mycelium.

From the study of the fungus in the above media it is found that some media are favourable for the formation of a large number of spores and some for the formation of chlamydospores; while some are good for both. A few media are unfavourable either for growth or formation of spores and chlamydospores. In some media spores are nearly of the same size as those found on the host under field conditions and in some they are much smaller. The perfect stage of the fungus does not occur on any of the above media, nor has it been found on the host.

(*d*) *The effect of reaction of media.* This experiment was run in duplicate using glucose agar. Twenty tubes were inoculated, two of each reaction, ranging from −15 to +30 Fuller's scale.*

TABLE 1.

Tube No.	Reaction	Growth after 12 days	REMARKS
1	−15 Fuller's scale	Poor	Growth poor but spore formation copious.
2	−10 ,,	Fair	In the beginning poor but after a few days fair. Spore formation normal.
3	−5 ,,	Fair	Growth was a little more than in −10.
4	0 ,,	Good	Aerial growth in sufficiently large amount.
5	+5 ,,	Very good	Aerial growth more than in 0.
6	+10 ,,	Very good	Aerial growth more than in 0.
7	+15 ,,	Good	The growth in the beginning was poor.
8	+20 ,,	Fair.	Aerial growth little.
9	+25 ,,	Poor	No spore formation.
10	+30 ,,	Very poo	In the beginning there was no growth. No spore formation.

* Media from +15 to +30 remained in the liquid condition on account of too much acidity.

These data show that this fungus prefers a reaction ranging between +5 and - 10 Fuller's scale, but can withstand a considerable range either way.

6. Etiology of the disease.

(*a*) *Formal proof of pathogenicity of Acrothecium Penniseti n. sp.* There is no doubt that this disease on *bajra* is due to this fungus as determined by the following points :—

1. The constant association of the fungus with the disease, and its isolation from typical diseased tissue of the host.

2. Healthy plants inoculated with pure cultures give characteristic signs of the disease. The penetration of the fungus was also noticed.

3. The fungus was reisolated from inoculated diseased spots and reinoculated on healthy plants and the disease was produced as before. The reisolated fungus was compared in culture with the fungus used for inoculation and was identical with it.

(*b*) *Details of inoculations.* The following are the details of the inoculations made on leaves and ears of *Pennisetum typhoideum* :

Experiment 1. In the beginning of September 1919, 26 inoculations were made by placing a little mycelium and spores from a pure culture on either side of a number of leaves and leaf-sheaths ; these were kept in a moist chamber. After 24 hours almost all the leaves were discoloured and had become pale in the places where they had been inoculated, and after 48 hours on microscopic examination it was found that the mycelium had penetrated in the tissues. Conidiophores and conidia started coming out after 72 hours. The infected spots gradually increased in size and were like those found in the field.

Experiment 2. (15-9-19.) Nine leaves of a healthy shoot were inoculated on either side with spores and mycelium from a pure culture as already described, and the shoot was covered with a bell jar. A control was also kept. On the fourth day four leaves showed signs of infection in the inoculated places, another spot appeared on the sixth day ; two more on the seventh, and the remaining on the eighth day. In the beginning the infected spots became pale and then gradually turned yellowish brown and ultimately dirty brown. All the spots increased in size and in five or six days after infection conidiophores began to come out. The control was intact.

Experiment 3. (15-9-19. Two healthy shoots with ears were inoculated in six places on leaves either side and in eight places on ears. A control was also kept. In this experiment one leaf took the infection within 48 hours, and on the fourth day three more showed infection. On the sixth day the remaining leaves were also found to be infected, showing the characteristic symptoms of the disease. All the inoculations made on spikelets of the ears were successful and the glumes became light brown. Conidiophores began to appear eight days after inoculations. The control was healthy.

Experiment 4. (18-9-19.) Twelve leaves and sheaths of a healthy young plant (about two months old) were inoculated on either side with spores and mycelium from

a young culture. The plant was covered with a bell jar. There was also a control. In 24 hours 7 leaves showed sign of infection and the spots inoculated showed penetration of hyphæ. After 48 hours three more were infected and two of the former spots had increased in size. On the fourth day infection took place on the remaining leaves and conidiophores began to appear four days later. The control showed no sign of disease.

Experiment 5. (26-9-19.) Three shoots with ears were placed in small flasks containing water. Nine inoculations on leaves and nine on the spikelets were made. Three shoots with ears were kept as controls. All inoculations on leaves and ears were successful on the third day. The spots on the leaves increased very much in size and those on the ears also increased and infected the neighbouring spikelets. There was no sign of disease either on leaves or on the ears of the controls.

Experiment 6. (26-9-19.) Ten inoculations on either side of the leaves and leaf-sheaths of two healthy young plants were made; spores only being used and the spots inoculated were covered with sterile cotton wool. The plants were placed outside in rain. A control was also kept. Six leaves took the infection in 24 hours and the remaining in 48 hours. The control remained healthy.

Experiment 7. (26-9-19.) Twelve leaves of two healthy full-grown plants were inoculated and kept in the same way as Experiment No. 6. A control was also kept. Four inoculations were successful in 24 hours, two on the third day and the rest on the fifth day. The control was without any sign of the disease.

Experiment 8. (11-10-19.) Twelve inoculations were made on four ears (3 on each) and the ears were covered with bell jars. A control was kept. After 48 hours a woolly growth appeared in the places inoculated and this went on increasing and infecting the neighbouring spikelets. Within ten days all the four ears were completely covered with aerial growth. The hyphæ penetrated the glumes and palæ, and in most cases even the ovary. The control was free.

Experiment 9. (21-10-19.) Three ears were inoculated as above and a control was also kept. The result was as in the above experiment.

Experiment 10. (25-10-19.) Three ears were inoculated in 9 places and the inoculated spikelets were covered with sterile moist cotton wool. After three days the cotton wool was removed and the spikelets were found to have turned brown in colour and on examination showed the presence of hyphæ inside them. The control that was kept showed no evident sign of the disease.

Experiment 11. (28-10-19.) Seventeen leaves and five ears of six healthy shoots were inoculated and the spots covered with sterile cotton wool. All except the control got infected in a week.

Experiment 12. (28-10-19.) Five leaves and three ears of a grown up plant were inoculated and the inoculated spots covered with sterile moist cotton wool. All inoculations on leaves and ears were so successful as to produce the characteristics of the disease. The control kept was satisfactory.

(c) ***Results of inoculations.*** Altogether 106 inoculations on leaves (both sides) and leaf-sheaths, together with 40 on ears, were made, and all were successful. The results of the above experiments prove beyond doubt the parasitic nature of *Acrothecium Pennisetí* on *Pennisetum typhoideum*.

TABLE II.

Summary of results of inoculations on Pennisetum typhoideum.

No. of experiment	Date	Where inoculated	Number of inoculations	Number infected	REMARKS
1	Beginning of September 1919.	Leaves	26	26	Leaves removed and kept in a moist chamber.
2	15-9-19	Leaves	9	9	Leaves on a shoot.
		Control	1	..	
3	15-9-19	Leaves	6	6	Leaves and ears on two healthy shoots.
		Ears	8	8	
		Control	1	..	
4	18-9-19	Leaves	12	12	Leaves on a plant.
		Control	1	..	
5	26-9-19	Leaves	9	9	
		Ears	9	9	Three shoots with ears.
		Controls	3	..	
6	26-9-19	Leaves	10	10	Two young plants.
		Control	1	..	
7	26-9-19	Leaves	12	12	Two mature plants.
		Control	1	..	
8	11-10-19	Ears	12	12	Four ears.
		Control	1	..	
9	21-10-19	Ears	3	3	Three ears.
		Control	1	..	
10	25-10-19	Ears	9	9	Three ears.
		Control	1	..	
11	28-10-19	Leaves	17	17	
		Ears	5	5	Six shoots with ears.
		Control	1	..	
12	28-10-19	Leaves	5	5	A mature plant.
		Ears	3	3	
		Control	1	..	

7. Relation of parasite to host.

The examination of inoculated leaves shows that infection can take place either through a stoma or through any epidermal cell on either side of the leaf.

(*a*) *Infection through a stoma.* The hypha near a stoma gives out a narrow tube which after passing through it again swells up and resumes its usual size. It spreads a little in the sub-stomatal space and then passes on to other cells (Plate IV, fig. 1).

(*b*) *Infection by piercing the epidermis.* The infection can take place from any cell of the epidermis but is more common from the thin-walled motor cells (Plate IV, figs. 2—4). The hypha either directly penetrates the epidermis or under the cuticle parallel to the outer wall of the epidermis to some extent, and then goes down either into an epidermal cell or between the side walls of two cells to cells deeper in. Sometimes when it is passing between the side walls of two cells, it sends a branch into one of them. Sometimes after inoculation the hyphæ are found in large amount under the cuticle but none in the cells below. Generally, before entering, the hypha coming in contact with the cuticle swells up and gives out a narrow tube which after penetrating the epidermis again swells up. Frequently the hypha presses down the cuticle of an epidermal cell to effect an entrance. After entering it may branch and fill the epidermal cell with a mass of mycelium or may directly penetrate deeply into the tissue of the leaf by passing from cell to cell and ramifying both in the thin-walled parenchymatous and the sclerenchymatous cells, and later may enter the vascular bundles.

It is both intra and inter-cellular. Haustoria are not found. In passing from cell to cell the hypha gives out a narrow tube which pierces the wall and on entering the opposite cell again resumes its former thickness. The fungus can invade new areas by passing along the vessels of the xylem (Plate IV, fig. 6). It cannot penetrate the midrib directly, but generally infection takes place from hyphæ passing in through the motor cells just on either side of it. The intra- and inter-cellular hyphæ are very clearly seen in the midrib region, and here sometimes they spread more through the inter-cellular spaces which are found at the angles of two or more cells (Pl. IV, fig. 5) than through the cells. Frequently the fungus forms stromatic masses in the epidermal cells on both sides of the leaf, sometimes in quantity sufficient to rupture the cuticle. In sclerenchymatous cells and in the vascular bundles hyphæ often swell up to such an extent so as to fill the cavity.

The fungus kills the cells in advance of its growth, and penetration of the dead cells is evidently easier than of the living, since in the central dead portion

of the spot hyphæ are found abundantly, but they are almost absent in the cells around the central dead portion.

After three or four days from infection conidiophores start coming out in clusters on both sides of the leaf through the stomata from a mass of mycelium lying in the sub-stomatal space. They even come out from the stomata on the midrib. When after inoculations infection is very vigorous they come out from the stromatic masses in the epidermal cells. It may be recalled here that in field conditions they always come out from the stomata.

8. Cross Inoculation experiments.

In order to find out whether this fungus can attack other hosts which have been also found infected by species of *Acrothecium*, inoculations were made on *Andropogon Sorghum* and *Zea Mays*.

Leaves and ears of *Andropogon Sorghum* were inoculated with the *bajra* fungus, but no infection took place.

A large number of inoculations were made on the leaves and male inflorescence of *Zea Mays*, and it was observed that the *bajra* fungus can infect the male inflorescence, but has no effect on the leaves.

A. lunatum, Wakker, which is found on *Andropogon Sorghum* leaves and male inflorescences of *Zea Mays* at Pusa, can infect to some extent, under laboratory conditions the young leaves of *Pennisetum typhoideum*.

9. Diagnosis.

The following is the diagnosis :—

Acrothecium Penniseti n. sp. Spots amphigenous on leaves and glumes ; in size generally 2—5 cm. long and 0·5—1 cm. broad ; dirty brown with yellowish margin. On the leaves more common along the edge and tip, and sometimes on the other parts including the midrib.

Fertile hyphæ rigid, erect, simple, 3—5 septate, straight or slightly bent ; fasciculate or solitary ; tip swollen or flexuous : olive brown to dirty brown with paler tips : base sometimes swollen. They are 68·4—145·4 μ long and 7·5—8·7 broad.

Conidia borne acrogenously on the conidiophore, forming a group of 2—5 spores ; clavate, pear-shaped or slightly bent, thick-walled, 2-3 septate, sometimes constricted at the septa ; olive brown to dirty brown ; 30·4—41·8μ long and 17·4—20μ broad. End cells lighter in colour ; middle cell broader and deeper in colour.

On leaves and ears of *Pennisetum typhoideum* in India.

10. Summary.

1. There are several species of *Acrothecium* known on various grasses, but none has hitherto been reported on *Pennisetum typhoideum*, and the present species does not agree with any of those previously described. It is a new disease caused by *Acrothecium Penniseti* n. sp.

2. It is found on ears, leaves and leaf-sheaths, and forms dirty brown spots with yellow margin.

3. Conidiophores arise in clusters through the stomata and spores are borne apically in fascicles of 2—5.

4. Infection can take place either through a stoma or by directly piercing an epidermal cell.

5. The fungus is cultivable on most artificial media but gives the highest development on wheat broth agar, potato juice agar, nutrient glucose agar, and on French-bean agar.

6. In culture secondary sets of conidia are formed either below the tip on the sides of conidiophore or above by the elongation of the latter.

7. In some media chlamydospores of a dirty brown colour are formed.

8. The growth is best on media having the reaction between +5 to +10 Fuller's scale but the fungus can withstand a wide range of reaction.

9. The parasitism of the fungus has been proved beyond doubt by numerous inoculations on leaves and ears.

10. The mycelium is both intra and inter-cellular and is found in all parts of the infected leaf.

11. Cross inoculations on male inflorescence of maize were successful while those on *Sorghum* gave negative results.

To Dr. E. J. Butler, Imperial Mycologist, under whose direction this work has been carried out, is due my grateful acknowledgment for his able advice and criticism.

APPENDIX.

1. Glucose meat-extract agar.

A	Peptone	1·0 grm.
	Sodium chloride	0·5 grm.
	Meat extract	0·5 grm.
	Glucose	4·0 grm.
	Water	100·0 c.c.
B	Agar agar	2·4 grm.
	Water	100·0 c.c.

2. Glycerine agar.

   It was prepared by adding 10 c.c. of pure glycerine to the above medium in place of glucose.

3. Litmus lactose agar.

   It was prepared by adding 2 grm. of c.p. lactose and 4 c.c. of blue litmus solution to medium No. 1 in place of glucose.

4. Starch agar.

   To 400 c.c. of hot water 5 grm. of pure starch were added and boiled for two hours. When a starch solution of 250 c.c. was obtained, it was added to 250 c.c. of the following nutrient solution.

A	Potassium phosphate	1 grm.
	Magnesium sulphate	1 grm.
	Sodium chloride	1 grm.
	Ammonium sulphate	2 grm.
	Water	100 c.c.
B	Calcium carbonate	1 grm.
	Water	900 c.c.

   A and B were mixed.

Starch solution	250 c.c.
Nutrient solution	250 c.c.
Agar	5 grm.

5. Dextrose agar.

Dextrose	2 grm.
Agar	3 grm.
Water	100 c.c.
Nutrient solution as for starch agar	100 c.c.

6. Cellulose agar.

Water	100 c.c.
Cellulose (Pure)	1 grm.
Nutrient solution as for starch agar ..	100 c.c.
Agar	2 grm.

7. Potato juice agar.

75 grammes of potato were cooked in a water bath for half an hour and the juice was filtered in a fine cloth, and to it was added 5 grammes of agar and sufficient water to make the whole 250 c.c.

8. Thaxter's hard potato agar.

125 grammes of sliced potato were cooked for half an hour in 500 c.c. of water. It was filtered in a fine cloth, and water was added to restore the original volume. To this was added 10 grammes of glucose and 15 grammes of agar and boiled for two hours, cleared, filtered and autoclaved.

9. Jowar (*Andropogon Sorghum*) agar.

One ounce of *jowar* seeds were powdered and boiled in 150 c.c. of water for two hours. The boiled mixture was filtered in a wire gauze strainer and to it 7·5 grm. of agar added and sufficient water to make the total 500 c.c.

10. Oat meal agar.

15 grammes of quaker oat meal were mixed in 200 c.c. of water and boiled. Filtered in cloth, squeezed out the content and to it 2 grammes of agar added.

11. French-bean agar (50–10–500).

50 grammes of powdered French-bean were boiled in water for one hour, filtered with wire gauze strainer and 10 grammes of agar added and water sufficient to make the total 500 c.c.

12. Corn meal agar.

13. Wheat meal agar.

14. Rice meal agar.

15. Barley meal agar.

All these were prepared as French-bean agar.

16. Jowar leaf juice agar (30–3–200).

30 grammes of leaves of *Andropogon Sorghum* were boiled for one hour in a water cooker. Filtered in a cloth and to it 3 grammes of agar added and water enough to make it to 200 c.c.

17. Maize leaf agar.

It was prepared as the above medium, only, in place of *jowar* leaves, *Zea Mays* leaves were used.

18. Wheat broth agar.

Agar	1·2 grm.
Wheat flour	150·0 grm.
Magnesium sulphate	0·5 grm.
Potassium nitrate	1·0 grm.
Glucose	5·0 grm.
Water	1000·0 c.c.

Filtered and sterilized on three consecutive days at 100°C.

19. Naegeli's nutrient solution.

Calcium chloride	0·1 grm.
Magnesium sulphate	0·2 grm.
Dipotassium phosphate	1·0 grm.
Ammonium tartrate	10·0 grm.
Distilled water	1000·0 c.c.

20. Cohn's nutrient solution.

Distilled water	1000·0 c.c.
Acid potassium phosphate	5·0 grm.
Magnesium sulphate	5·0 grm.
Neutral ammonium tartrate	10·0 grm.
Potassium chloride	0·5 grm.

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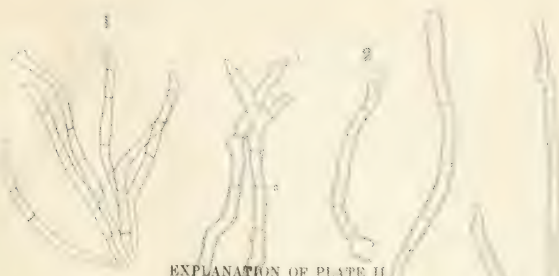
ACROTHECIUM ON PENNISETUM TYPHOIDEUM.



EXPLANATION OF PLATE II

Fig. 1. Conidiophores from the host in a cluster (×490).

„ 2. Six detached conidiophores from an infected leaf. (*a*) Conidiophor forked near the tip (×750).

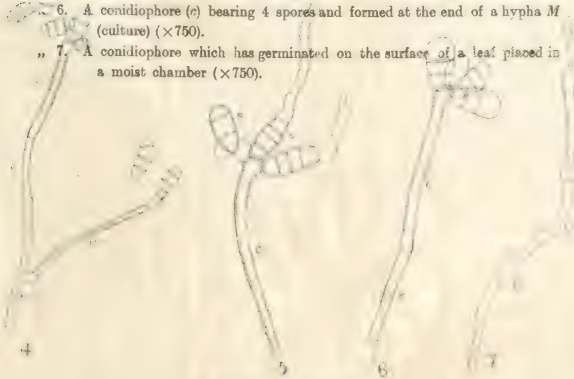
„ 3. Spores from an infected leaf. (*a*) Germination of a spore after one and a half hours from placing in distilled water (×750).

„ 4. A portion of mycelium from a culture, with conidiophores and conidia. *M*. Sterile portion of the mycelium. (*c*) A conidiophore bearing 2 spores at its tip, one of which is shown detached. (*c*) A conidiophore formed at the end of a hypha bearing 5 spores at its tip, 3 of which are shown detached (×490).

„ 5. Germination of 2 spores (*s*) which are still attached to the tip of a conidiophore (*c*). (*s*) A spore which has begun to germinate from the end by which it was attached (culture) (×490).

„ 6. A conidiophore (*c*) bearing 4 spores and formed at the end of a hypha *M* (culture) (×750).

„ 7. A conidiophore which has germinated on the surface of a leaf placed in a moist chamber (×750).



EXPLANATION OF PLATE II.

Fig 1. Conidiophores from the host in a shoot [illegible]

" 2. Six detached conidiophores from an infected leaf [illegible] conidiophore forked near the tip (× 750).

" 3. Spores from an infected leaf. (a) Germination of a spore after one and a half hours from placing in distilled water [illegible]

" 4. A portion of mycelium from a culture, with conidiophores and conidia. M. Sterile portion of the mycelium. (a) A conidiophore bearing 2 spores at its tip, one of which is shown detached. (c) A conidiophore bearing at the end of a hyphа bearing 5 spores at its tip, 4 of which are shown detached (× 750).

" 5. Germination of 2 spores (a) which are still attached to the tip of a conidiophore (c). (a) A spore which has begun to germinate from the end of which it was attached (culture [illegible] × 750).

" 6. A conidiophore (c) bearing 4 spores and formed at the end of a hyphа M (culture) (× 750).

" 7. A conidiophore which has germinated on the surface of a leaf placed in a moist chamber (× 750).

ACROTHECIUM PENNISETI.

EXPLANATION OF PLATE III.

Fig. 1. Formation of chlamydospore (mature) in nutrient glucose agar (×750).
„ 2. A hypha from glucose agar showing the beginning of the formation of chlamydospores (×750).
„ 3. Same as above but a little more advanced stage is shown (×750).
„ 4. A portion of the mycelium from the same culture from which Figs. 2 & 3 were drawn (×750).
„ 5 Three conidiophores from a glucose culture, one of them is forked near the tip (×750).
Figs. 6—10. Germination of spores from a culture (×750).
Fig. 11. Spores from a culture showing different forms and septation (×750).

EXPLANATION OF PLATE III

Fig. 1. Formation of chlamydospore (mature) in nutrient gelatine agar ; × 750.

„ 2. A hypha from glucose agar showing the beginning of the formation of chlamydospores ; × 750.

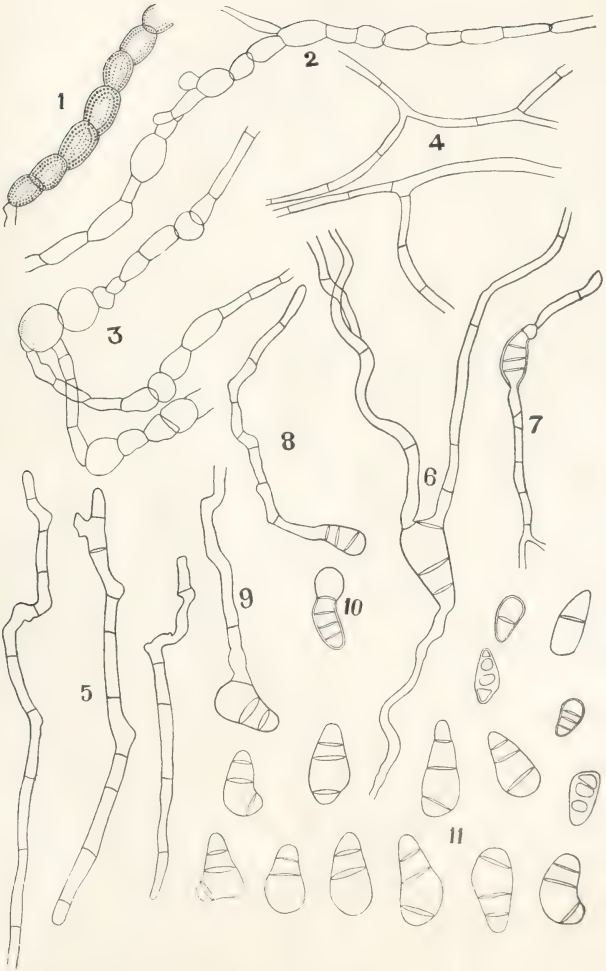
„ 3. Same as above but a little more advanced stage is shown ; × 750.

„ 4. A portion of the mycelium from the same culture from which Figs. 2 & 3 were drawn ; × 750.

„ 5. Three conidiophores from a glucose culture. One of them is dotted near the top ; × 750.

Figs. 6—10. Germination of spores from a culture ; × 750.

Fig. 11. Spores from a culture showing different forms and segmentation ; × 750.



ACROTHECIUM PENNISETI.

EXPLANATION OF PLATE IV.

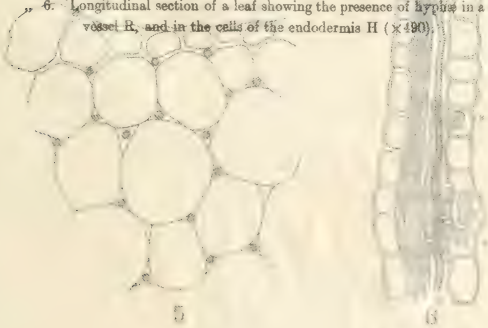
Fig. 1. Section of an inoculated leaf showing the penetration of a hypha through a stoma. (×750).

„ 2. Section of an inoculated leaf showing the penetration through the cuticle directly into an epidermal cell (×750).

Figs 3 & 4. Sections of an inoculated leaf from different places showing the presence of hyphæ under the cuticle after penetration and in some places forcing their way through the side walls of two epidermal cells into cells deeper in (×490).

Fig. 5. Section of the midrib (upper side) of an infected leaf showing hyphæ in the inter-cellular spaces (×750).

„ 6. Longitudinal section of a leaf showing the presence of hyphæ in a reticulate vessel R, and in the cells of the endodermis H (×490).



EXPLANATION OF PLATE IV.

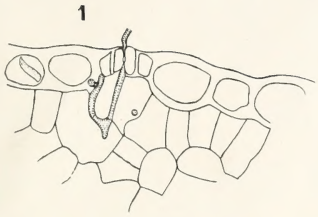
Fig. 1. Section of an inoculated leaf showing the penetration of a hyphæ through a stoma (×750).

„ 2. Section of an inoculated leaf showing the penetration through the cuticle directly into an epidermal cell (×750).

Figs 3 & 4. Sections of an inoculated leaf from different places showing the presence of hyphæ under the cuticle after penetration and in some places forcing their way through the side walls of two epidermal cells into cells deeper in (×430).

Fig. 5. Section of the midrib (upper side) of an infected leaf showing hyphæ in the inter-cellular spaces (×750).

„ 6. Longitudinal section of a leaf showing the presence of hyphæ in a reticulate vessel R, and in the cells of the endodermis E (×750).



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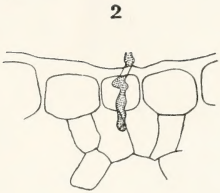

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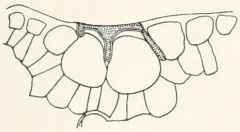

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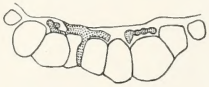

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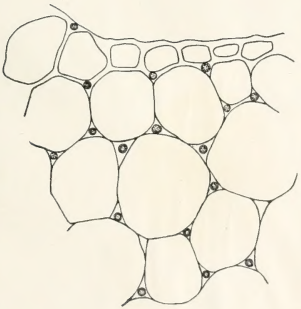

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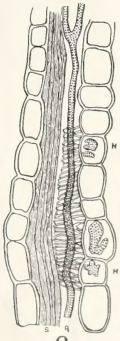

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ACROTHECIUM PENNISETI.